Journal of Coastal Research	28	4	780–786	West Palm Beach, Florida	July 2012

Landscape Dynamics Determine the Small-Scale Genetic Structure of an Endangered Dune Slack Plant Species

Dries Bonte[†], Peter Breyne[‡], Rein Brys[†‡], Eduardo de la Peña[†], Bram D'hondt[†], Céline Ghyselen[†], Martijn L. Vandegehuchte[†], and Maurice Hoffmann[†‡]

[†]Ghent University
Department of Biology
Terrestrial Ecology Unit
K.L. Ledeganckstraat 35
9000 Ghent, Belgium
Dries.Bonte@Ugent.be

[‡]Research Institute for Nature and Forest
Department of Biodiversity and Natural Environment
Kliniekstraat 25
1070 Brussel, Belgium

www.cerf-jcr.org

www.JCRonline.org

ABSTRACT

Bonte, D.; Breyne, P.; Brys, R.; de la Peña, E.; D'hondt, B.; Ghyselen, C.; Vandegehuchte, M.L., and Hoffmann, M., 2012. Landscape dynamics determine the small-scale genetic structure of an endangered dune slack plant species. *Journal of Coastal Research,* 28(4), 780–786. West Palm Beach (Florida), ISSN 0749-0208.

Understanding the processes that determine genetic variation within landscapes is a crucial factor for successful management of threatened plant species that are sensitive to both environmental and genetic bottlenecks. While current insights point to the importance of historical landscape processes for the genetic structure of populations at large spatial scales, their relevance at small spatial scales has been largely neglected. In this context, coastal dunes are a typical example of dynamic and geologically young landscapes in which current and historical sand drift may have strong impacts on the spatial dynamics of a large number of plant species. One of these is the endangered plant species *Parnassia palustris*, typically inhabiting dune slacks formed by recent sand displacements in parabolic dune landscapes. Dune slacks originating from the same sand drift process are located within the same parabola unit. The species is known to suffer from dispersal limitation and from inbreeding when genetic exchange between populations is restricted. By means of amplified fragment length polymorphism, we demonstrate that the species shows a genetic substructuring both at the level of the metapopulation and the local landscape. Populations located within the same parabola unit are much more closely related than expected on the basis of geographic distance. Moreover, population size is related to genetic diversity within populations. The species' population genetic structure should consequently be regarded as a shifting mosaic of genetic variation, mediated by sand drift driven landscape formation. Therefore, the maintenance of sand dynamics is essential to preserve genetic diversity in dynamic dune landscapes.

ADDITIONAL INDEX WORDS: *AFLP, metapopulation,* Parnassia palustris*, population size, sand dynamics.*

INTRODUCTION

Understanding the processes and patterns of gene flow and local adaptation requires a detailed knowledge of how landscape characteristics affect the genetic structure of populations (Holderegger and Wagner, 2008). This is especially true for plants, in which the process of gene flow is often strongly influenced by the configuration of the surrounding landscape and prevented by the barriers within it (Sork *et al.,* 1999). This understanding is crucial, not only to improve general insights on genetics in conservation biology, but also to properly manage endangered species in order to maintain and restore the genetic diversity of their populations. Approaches from so-called landscape genetics (Holderegger and Wagner 2008) promise to facilitate our understanding of how geographic and environmental features may affect genetic variation at both the population and individual level and may have important implications for ecology, evolution, and conservation biology (Manel *et al.,* 2003). Recent population genetic studies have demonstrated the relationship between population size, distance between populations, and genetic differentiation among populations, which is strongly affected by the species' set of life history traits (see, *e.g.,* Hamrick and Godt, 1996; Honnay and Jacquemyn 2007; Nybom and Bartish, 2000 for comprehensive reviews). In contrast, only few studies have explicitly tested how landscape genesis (*i.e.,* processes that shape the landscape configuration) may affect spatial patterns in neutral genetic variation, underlying microevolutionary processes, and the eventual feedback on life history traits (Balkenhol *et al.,* 2009; Storfer *et al.,* 2007).

In general, studies aiming to test the relative importance of historical *vs.* current gene flow in relation to landscape genesis are constrained by the fact that the configuration of most landscapes to date is heavily influenced by anthropogenic factors on a very short timescale relative to most natural processes of landscape formation. Therefore, the observed genetic variation of these populations often does not confirm general predictions of (meta-) population genetic theory, for instance, the fact that genetic distance and geographic distance are not always found to be related (Bonnin *et al.,* 2002; Leimu *et al.,* 2006).

DOI: 10.2112/JCOASTRES-D-10-00128.1 received 24 August 2010; accepted in revision 1 November 2010.

Landscape formation may induce spatial genetic variation due to historic gene flow and common founder effects and by decreasing local population sizes if habitat formation becomes restricted. Two consequences of small population size are increased genetic drift and inbreeding. Genetic drift and inbreeding may influence small plant populations by changing patterns of genetic diversity. Genetic drift decreases genetic variation within populations, while it generally increases differentiation among populations, an effect that becomes more pronounced in declining populations (Barrett and Kohn, 1991; Ellstrand and Elam, 1993; Young, Boyle, and Brown, 1996). Inbreeding increases homozygosity within populations, whereas smaller populations lose genetic variation faster than larger populations (Ellstrand and Elam, 1993). The expected relationship between population size and genetic diversity is, however, often missing (Bonnin *et al.*, 2002; Leimu *et al.,* 2006) because populations occur in human managed habitats that could have experienced dramatic short-term changes. As Bonnin *et al.* (2002) suggest, these changes may include bottlenecks and a changing degree of connection to other populations (Holderegger and Wagner, 2008).

In dynamic landscapes, landscape genesis is by definition a geologically young process that can often be disentangled from human interference on habitat configuration. Coastal dunes along the North Sea comprise such a system. Owing to sand drift dynamics, dry parabolic dune ridges are continuously shifting in a northeasterly direction, leaving behind young ephemeral dune slacks (Provoost, Jones, and Edmondson, 2009), whose ecological characteristics are often determined by the groundwater level and the carbonate content at the moment of development. Here, several plant species find their optimal, though temporary, growing conditions; they are often restricted to specific environmental conditions in space and time. Their populations are subsequently expected to be predominantly linked to the historical dynamics of these landscapes, in which gene flow patterns are strongly determined by the occurrence of dry parabolic dune ridges that may function as effective dispersal barriers. On the other hand, the fragmentation of larger dune entities due to the steady increase of urbanized areas is another factor that may induce genetic variation at larger geographic scales. So, the historical intact and connected coastal dune region has been fragmented by urbanization into several entities, further referred to as the *metapopulations*. Separated *populations* occur in dune slacks, which are due to common history situated in larger *parabola units*. Populations are subsequently hierarchically located in parabola units situated within one metapopulation (*i.e.,* the dune entity).

Bonnin *et al.* (2002) analyzed the population genetic structure of 14 *Parnassia palustris* populations in northern France. They compared populations occurring in different habitats and found restricted gene flow among populations. However, panmixia within eight of the populations suggested that, in contrast to seed dispersal, pollen transfer was not limited at a regional scale. In contrast to the populations studied by Bonnin *et al.* (2002), the studied *P. palustris* populations in this work are closely located in isolated metapopulations within a small region along the Flemish–French coast. A pollination experiment, with outside-metapopulation crosses resulting in a higher reproduction capacity than within-metapopulation crosses, indicated a larger degree of compatibility between plants originating from different metapopulations (Bossuyt 2007). By means of amplified fragment length polymorphism (AFLP) analysis, we tested the hypothesis that landscape dynamics (*i.e.,* parabolic dune genesis) are important drivers of genetic differentiation in *P. palustris* within existing metapopulations relative to among-metapopulation differentiation.

MATERIAL AND METHODS

Study Species

Parnassia palustris (Saxifragaceae) is a perennial herb with a circumboreal distribution (Bonnin *et al.,* 2002; Borgen and Hultgård, 2003). Each plant has basal leaves and 1–30 flowering stems, each with one terminal flower. The species is hermaphroditic and protandrous. The five stamens sequentially discharge their pollen before the stigma becomes receptive. It is usually cross-pollinated and rarely autogamously pollinated (Martens, 1936). The species is mainly outcrossing and strongly depends on pollinators for optimal pollination and seed set (Sandvik and Totland, 2003). *Parnassia palustris* is insect pollinated, mainly by Diptera, particularly hoverflies (Syrphidae), but other insects contribute to pollen transfer (Sandvik and Totland, 2003). It flowers from August through September, and fruits, containing several hundreds of seeds, ripen from September through October. The small, light seeds are dispersed by water and wind. *Parnassia palustris* is considered endangered in northern France, Luxembourg, Belgium, and the Netherlands (Bonnin *et al.,* 2002), where it is a rare plant in lime-rich dune slacks along the coast and inland lime-rich marshes.

Study Area

The study was conducted in a sandy dune area along the western Belgian and northeastern French coast. This area has been extensively fragmented by human activities since the beginning of the 20th century. The remaining dune areas are protected as nature reserves and managed by mowing and extensive grazing. Within these landscapes, dune slacks are frequently formed by wind erosion down to the level of the groundwater at the backside of moving dune ridges, termed parabolic dunes. Such dune slacks, with a calcareous and nutrient-poor soil, flood in winter, dry up in summer, and form a temporary natural habitat for *P. palustris*. *Parnassia palustris* populations are consequently restricted to individual parabolic units, each of these representing separate landscape entities with a common history of origin. Populations of *P. palustris* only persist in the relatively early-successional stages of vegetation establishment within these dune slacks. Further succession leads to dune shrub formations. However, the mowing of shrub encroached dune slacks may also result in the potential persistence of *P. palustris* populations. Both natural parabola dynamics and human conservation actions may thus determine the spatial structure of the species in the remaining intact dune areas (further referred to as metapopulations; see Bossuyt 2007; Bossuyt and Honnay 2006;

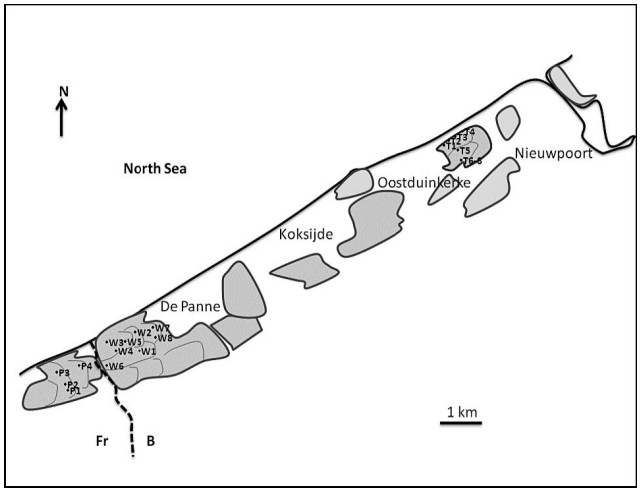

Figure 1. Location of the sampled *P. palustris* populations along the coast of Flanders (B) and Northern France (Fr). P, Perroquet populations; W, Westhoek populations; T, Ter Yde populations. Light blue lines indicate parabolic dunes, shifting to the northeast, leaving behind dune slacks on the weather side. In blue: remaining dune areas along the coast.

Table 1. *Genetic diversity and population characteristics of the studied* P. palustris *populations (*n*, number of sampled plants for population genetic analysis; PPL, percentage of polymorphic loci;* H_j*, expected genetic diversity). The symbols in the population ID refer to the samples metapopulation: P, Perroquet; T, Ter Yde; W, Westhoek.*

Population	*n*	PPL	H_j	Population Size	Patch Area (m^2)
P1	19	83.80	0.32	200	2808
P2	18	85.30	0.34	180	2466
P3	18	79.40	0.31	120	2342
P4	17	82.40	0.32	100	723
T1	19	88.20	0.33	50,000	4105
T2	19	82.40	0.32	300	2506
T3	10	82.40	0.32	125	2596
T4	20	86.80	0.34	220	11,070
T5	20	86.80	0.35	1400	4798
T6	7	80.90	0.31	14	413
T7	10	79.40	0.29	10	234
T8	10	82.40	0.30	27	263
W1	18	85.30	0.35	2400	9064
W2	17	89.70	0.32	350	10,227
W3	19	86.80	0.31	40,000	5196
W4	16	85.30	0.32	3200	960
W5	17	83.80	0.33	700	3910
W6	18	88.20	0.34	2400	23,953
W7	19	91.20	0.35	125	1547
W8	9	83.80	0.32	9	821

Bossuyt, Honnay, and Hermy, 2003). *Parnassia palustris* consequently experiences two levels of fragmentation along this coast: large-scale fragmentation due to urbanization, and small-scale fragmentation due to the combined action of parabolic dune dynamics, shrub encroachment, and mowing management.

Based on the study of Bossuyt (2007), we selected 20 populations of *P. palustris* within three metapopulations (Figure 1). Each population is thus restricted to one dune slack. In the Ter Yde metapopulation (T, 260 ha) we sampled eight populations, ranging in size from three to more than 10,000 individuals. All plants were randomly selected in the population, so both at the periphery and the center. This metapopulation is separated from the two other metapopulations, Westhoek (W, 340 ha) and Perroquet (P, 225 ha), by a densely urbanized area of 10 km where no individuals of *P. palustris* occur. The metapopulations W and P are separated by a road and a camping site at the border between Belgium and France. We sampled eight and four populations in the W and P metapopulations, respectively, ranging in size between seven and more than 10,000 individuals. An overview of the sampled populations is given in Table 1.

AFLP Analysis

Leaves sampled in the field were immediately frozen in liquid nitrogen. In the lab, they were freeze-dried for 48 hours and homogenized with a mill (Retsch MM 200) to fine powder. We used 20 mg of dried leaf material for DNA extraction using the QuickPick™ plant DNA kit (Isogen Life Science). DNA quality was checked on 1.5% agarose gels. Concentration and purity were determined using a ND-1000 spectrophotometer (NanoDrop, Thermo Scientific). We used 100 ng of DNA for AFLP analysis according to Vos *et al.* (1995). Restriction and ligation were done in a single step. Amplification of fragments was performed in two steps using the primer combinations EcoRI+A/MseI+C and EcoRI+C/MseI+G for preamplification and EcoRI+ATC/MseI+CAT, EcoRI+ACA/MseI+CAC, and EcoRI+CCA/MseI+GTT for selective amplification. Fragment separation and detection took place on a Nen IR² DNA analyzer (Licor) using 24 cm denaturing gels with 6.5% polyacrylamide. IRDye size standards (50 to 700 bp) were included for sizing of the fragments. Control samples were included in each gel to check for reproducibility within and between gels. Only clear, intense polymorphic bands between 75 bp and 500 bp were scored. Scoring was done using the SAGAmx software (Licor). We scored the presence or absence of every marker in each individual as 1 or 0 (present or absent) to form a binary data matrix.

Genetic Data Analysis

Based on the allele frequencies, within-population and metapopulation genetic diversity was estimated by the percentage of polymorphic loci (PPL) and Nei's genetic diversity (expected heterozygosity, H_j). Additionally, we determined the proportion of total genetic variability within a population compared to the total genetic variability recorded among the three metapopulations (population differentiation, F_{ST}) and total metapopulation diversity (H_t; Lynch and Milligan, 1994). The number of permutations for the test on F_{ST} was 999. These measures were calculated using AFLP-Surv (Vekemans *et al.,* 2002). To assess the degree of molecular variation within and among populations, total genetic diversity was partitioned by applying a hierarchical analysis of molecular variance (AMOVA; Table 2) on Euclidean pairwise genetic distances using Genalex 6.1 (Peakall and Smouse, 2006). Significances were determined based on 999 permutations. The $Ø_{ST}$ is an

Table 2. *Hierarchical partitioning of the genetic variation among metapopulations (MP). For each source of the variation (within populations, among populations from the metapopulation, and among metapopulations), we provide the statistical attributes from the MANOVA (df, degrees of freedom; SS, sum of squares; MS, mean squares; Est. Var., estimated variance component; and variance explained). The different variance contributions are denoted by specific genetic statistics (Stat) that are tested for their significance level (Value, Prob).*

Source	df	SS	MS	Est. Var.	Variance Explained	Stat	Value	Prob
Among MP	2	375.191	187.595	1.471	13%	$Ø_{RT}$	0.125	0.001
Among pops/MP	17	440.178	25.893	0.997	8%	$Ø_{PR}$	0.097	0.001
Within pops	317	2946.807	9.296	9.296	79%	$Ø_{PT}$	0.210	0.001
Total	336	3762.175	222.784	11.763				

analogue for F_{ST} values used for dominant markers such as AFLP and was derived from the Euclidean genetic distances. Its significance was calculated using the Monte Carlo procedure in Genalex 6.1 (999 permutations). Pairwise genetic distances among the populations and their level of significance were obtained from AMOVA. Again 999 permutations were applied. The relationship between pairwise genetic distances (F_{ST}), derived from AFLP-Surv and geographic distances (Table 3), was assessed with Mantel tests implemented within the ade4 package of R statistical software v.2.6.0 (R Development Core team; 999 replicates). Similarly, differences in pairwise genetic distances (F_{ST}) between populations from the same or different parabola entities were tested by a distance-based nested permutational analysis of variance (PERMANOVA).

RESULTS

Population Genetic Structure

The three AFLP primer combinations rendered 68 highly reliable polymorphic markers. Genetic diversity within populations was high, with the percentage of polymorphic loci (PPL) ranging from 79.4 to 91.2. Concordantly, the expected heterozygosity (H_j) ranged from 0.29 to 0.35. There were no obvious differences in genetic diversity levels between the three metapopulations. Total genetic diversity (H_t) reached 0.364, while the overall level of genetic differentiation (F_{st}) was 0.11 ± 0.08 and highly significant, indicating substantial genetic substructure. This is confirmed by partitioning (Table 2) of the total genetic variation among the metapopulations (MANOVA, $Ø_{RT} = 0.125$, $P < 0.001$). Genetic variation within each of the three metapopulations was lower, but substantial and significant (among-population variation, MANOVA, $Ø_{PR} = 0.097$, $P < 0.001$), while the largest amount of variation was due to individual variation within each population (within-population variation, MANOVA, $Ø_{PT} = 0.210$, $P < 0.001$). Genetic diversity within populations subsequently accounted for nearly 80% of the observed genetic variation. Variation among and within metapopulations was 13% and 8%, respectively. When considering all populations, genetic differentiation is driven by isolation by distance (Mantel test, $r = 0.632$, $P < 0.001$).

Within-Metapopulation Genetic Diversity

Within metapopulations, genetic differentiation is apparently not driven by isolation by distance (Mantel test for Ter Yde [T], $r = 0.0147$, $P = 0.434$; Westhoek [W], $r = -0.413$, $P = 0.991$; Perroquet [P], $r = 0.223$, $P = 0.421$). For all three metapopulations, the partitioning of the genetic diversity is largest at the within-population level (MANOVA; Table 3). For W, T, and P, 9%, 6%, and 13% of the diversity can be attributed

Table 3. *Hierarchical partitioning of the genetic variation within each of the metapopulations, so disentangling within populations, among populations from each parabola unit, and among parabola units from the specific metapopulation (Westhoek, Ter Yde, Perroquet). We provide the statistical attributes of the MANOVA (df, degrees of freedom; SS, sum of squares; MS, mean squares; Est. Var., estimated variance component, and variance explained). The different variance contributions are denoted by specific genetic statistics (Stat) that are tested for their significance level (Value, Prob). Partitioning of the genetic diversity within each of the three metapopulations.*

Source	df	SS	MS	Est. Var.	Variance Explained	Stat	Value	Prob
Westhoek (W)								
Among parabolas	2	56.810	28.405	0.034	0%	$Ø_{RT}$	0.003	0.233
Among pops/parabola	5	132.533	26.507	0.969	10%	$Ø_{PR}$	0.092	0.001
Within pops	134	1274.279	9.510	9.510	90%	$Ø_{PT}$	0.095	0.001
Total	141	1463.622	64.421	10.512				
Ter Yde (T)								
Among parabolas	1	27.728	27.728	0.156	2%	$Ø_{RT}$	0.016	0.001
Among pops/parabola	6	108.049	18.008	0.608	6%	$Ø_{PR}$	0.062	0.001
Within pops	111	1025.156	9.236	9.236	92%	$Ø_{PT}$	0.076	0.001
Total	118	1160.934	54.972	9.999				
Perroquet (P)								
Among parabolas	1	46.403	46.403	0.364	3%	$Ø_{RT}$	0.034	0.004
Among pops/parabola	2	68.655	34.327	1.358	13%	$Ø_{PR}$	0.131	0.001
Within pops	72	647.371	8.991	8.991	84%	$Ø_{PT}$	0.161	0.001
Total	75	762.429	89.721					

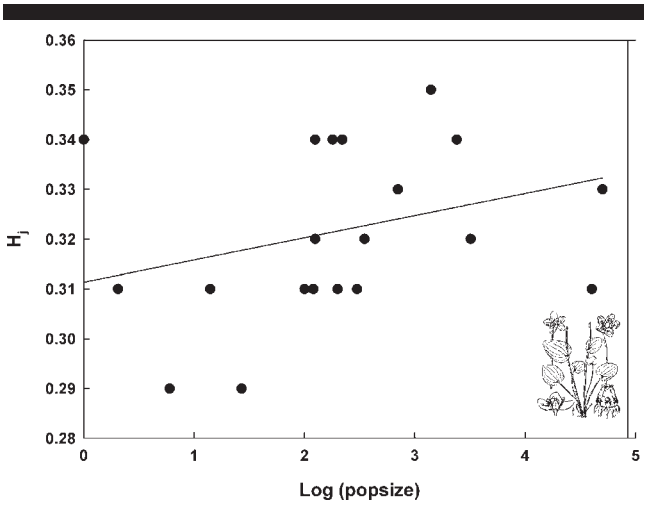

Figure 2. Relationship between expected heterozygosity within each of the sampled *P. palustris* populations and population size, based on linear regression (see text).

to the population level within parabola unit, respectively. Among these parabola units, however, variation between populations is low or nonexistent.

The genetic substructuring is, nevertheless, largely determined by landscape formation, with populations within the same parabola units showing higher levels of genetic similarity compared with those from different units. This is reflected by the outcome of the PERMANOVA, in which significantly different pairwise F_{st} values between dune areas were found (pseudo $F = 11.116$, $P = 0.025$) and between parabola units within the three different metapopulations (pseudo $F = 2.221$, $P = 0.038$).

In addition to the mentioned differentiation between parabola units, within-metapopulation differentiation may also be driven by drift and the loss of genetic variation in small populations. This is evidenced by the positive relationship between within-population genetic diversity and population size as revealed by mixed model regression ($\beta = 0.008 \pm 0.003$ SE, $F_{1,17} = 4.63$, $P = 0.047$) and correlation ($r = 0.34$, $P < 0.05$; see Figure 2) and the fact that the number of pairwise differentiations with other populations from the same metapopulation decreases with population size ($r = -0.680 \pm 0.255$ SE, Wald $\chi^2 = 7.19$, df = 1, $P = 0.0073$), when corrected for differences in average genetic differentiation between the three metapopulations (df = 2, Wald $\chi^2 = 12.06$, $P = 0.0024$). No differences in genetic diversity within populations were found between metapopulations (effect of metapopulation and metapopulation $\times$ log [population size], both $F_{2,13} < 2.18$, $P > 0.05$). Because population sizes differ neither between parabola units ($F_{5,11} = 1.17$, $P = 0.343$) nor between the different metapopulations ($F_{2,11} = 1.90$, $P = 0.195$), differences in population size do not underlie the retrieved differentiation between parabola units.

DISCUSSION

This study clearly indicated that the genetic structure of our studied coastal dune populations of *P. palustris* is shaped by isolation by distance at larger geographical scales. At smaller spatial scales, genetic substructuring according to the landscape unit (*i.e.,* the parabola) rather than by geographic distance was observed. The loss of genetic variation in small populations and drift induced additional differentiation among populations. The species' level of variation is in line with studies on species with similar life history at a similar spatial scale (Hamrick and Godt, 1996; Honnay and Jacquemyn 2007; Nybom and Bartish, 2000). However, since genetic differentiation increases with geographic distance (Nybom and Bartish, 2000), our reported F_{st} values are lower than those recorded in studies at larger regional or even continental scales (see for instance Bonnin *et al.,* 2002).

Our results point to the importance of considering landscape genesis in order to understand historical and contemporary gene flow patterns in plant populations. Although this is indeed previously acknowledged for plants by the seminal paper of Sork *et al.* (1999), empirical studies at this small scale in terrestrial systems are scarce (but see Honnay *et al.,* 2009). Some studies explicitly testing how river bank geometry and dynamics impact the genetic structure in plants (Honnay *et al.,* 2009; Kondo, Nakagoshi, and Isagi, 2009; Kudoh and Whigham, 1997; Van Looy *et al.,* 2009) and arthropods (Lambeets, Breyne, and Bonte, 2010) showed that "isolation by functional (dis)connectivity" rather than "isolation by distance" is a more important driver for genetic diversity. Our study subsequently acknowledges this approach, even at small spatial scales.

AFLP analysis revealed isolation by distance when considering the three metapopulations, or the combined Westhoek–Perroquet metapopulation, but not when contrasting genetic with geographic distance within each of the single metapopulations. These observations are in contrast with the study of Bonnin *et al.* (2002), who did not find any sign of isolation by distance even at larger spatial scales by using allozyme or cpDNA markers. While the different genetic techniques may be responsible for the contradicting patterns observed, the overall landscape context of the study is probably a more tentative alternative explanation. Bonnin *et al.* (2002) studied 14 French populations in different contrasting habitat types with a long history of fragmentation due to landscape formation at large geological scales (chalk hills, marshes, old and young dune systems). In contrast, our study emphasized patterns of genetic diversity within a young coastal dune landscape that only experienced fragmentation due to urbanization after the Second World War (Provoost *et al.,* 2004). The combined action of genetic drift, natural selection, and limited gene flow between distantly isolated *P. palustris* populations is therefore suggested to destroy any signature of isolation by distance in Bonnin's French populations, while this may not be the case in our study area. This is additionally confirmed by the higher levels of genetic differentiation in the studied populations of Bonnin *et al.* (2002), both with isozyme and cpDNA markers.

The genetic substructuring within each of the metapopulations, according to the landscape history (and parabola formation), suggests that gene flow is restricted among parabola units. It is, however, remarkable that nearby populations of different parabola units, even at the opposite side of a parabola dune unit, are genetically more different than distant populations within the same parabola unit. These dune

ridges often reach up to more than 10 m above the slacks and are partly vegetated with shrubs. This suggests that these parabola dunes may act as efficient gene flow barriers, either for pollen or seeds. The tiny seeds of *P. palustris* are well adapted to wind or water dispersal (Bouman *et al.,* 2000; Sandvik and Totland 2003). Because coastal dune slacks inundate during winter, dispersal by water during the winter inundation may be responsible for gene flow among some populations from the same parabola unit and constrain seed dispersal among parabola units that are separated by higher dune ridges, acting as watersheds.

Although similar within-parabola dispersal may be expected for wind dispersal, it is less obvious for pollen transfer. According to Ennos (1994), pollen migration contributes 20 times more to gene flow among populations than seed migration in *P. palustris* (if in mutation-drift equilibrium). Personal observations of one of us indicated that *P. palustris* is predominantly pollinated by the hover fly *Episyrphus balteatus* (Degeer). In a study in cultivated landscapes, Wratten *et al.* (2003) found field boundaries to be barriers for pollen transfer with a serious decline over distances of 200 m, suggesting that even common pollinators like *E. balteatus* may have a limited impact on pollen transfer in structured landscapes with pronounced geomorphological barriers.

Because *P. palustris* has been shown to be sensitive to the loss of genetic variation within and among metapopulations (Bossuyt 2007), resulting in a fitness decline due to the partial self-incompatibility, conservation of genetic diversity and gene flow within and among metapopulations appears to be essential for the maintenance of population viability in the long term. Bossuyt (2007) found no evidence for outbreeding depression on seed set when crossing plants from different metapopulations, but, of course, it could manifest after several generations. Our study showed that the population structure should be regarded as a shifting mosaic of genetic variation, affected by sand drift–mediated landscape formation. Outcrossing with genetically different pollen enhances fitness (Bossuyt 2007). The conservation of this within-metapopulation genetic variation due to the maintenance of natural sand drift, in combination with some gene flow among parabola dunes, is therefore likely to be an important attribute to maintain the species' fitness at the highest level. Bossuyt (2007) showed that especially small populations benefited from outcrossing with genetically different pollen. Consequently, in addition to restoring large population sizes, *P. palustris* may particularly benefit from genetic rescue. An increased genetic variation within both local populations and metapopulations is therefore very likely to be advantageous for population persistence, especially because high estimates of genetic differentiation suggest that gene flow among metapopulations is disrupted as a result of high levels of urbanization. This study therefore adds evidence on the importance of natural sand dynamics for the conservation of genetic variation within metapopulations in addition to the positive effects of natural sand dynamics for the conservation of biodiversity at the species level (Bonte, Maelfait, and Lens, 2006; Maes and Bonte 2006) and at the level of ecological functioning (Bonte, Maelfait, and Lens, 2006; Heidinger, Hein, and Bonte, 2010; Vandegehuchte, de la Peña, and Bonte, 2010).

ACKNOWLEDGMENTS

We thank Bea Bossuyt for help with the sampling of *Parnassia* material, thus providing a solid basis for this paper. Bram D'Hondt, Céline Ghyselen, and Martijn Vandegehuchte are Ph.D. students funded by the Research Foundation—Flanders (FWO). Eduardo de la Peña and Rein Brys are postdoctoral fellows funded by the FWO. Dries Bonte is supported by the EVE-NET FWO-research network.

LITERATURE CITED

Balkenhol, N.; Gugerli, F.; Cushman, S.A.; Waits, L.P.; Coulon, A.; Arntzen, J.W.; Holderegger, R., and Wagner H.H., 2009. Identifying future research needs in landscape genetics: where to from here? *Landscape Ecology,* 24, 455–463.

Barrett, S.C.H. and Kohn, J., 1991. The genetic and evolutionary consequences of small population size in plant: implications for conservation. *In:* Falk, D. and Holsinger, K.E., (eds.), *Genetics and Conservation of Rare Plants.* Oxford, U.K.: Oxford University Press, pp. 3–30.

Bonnin, I.; Colas, B.; Bacles, C.; Holl, A.C.; Hendoux, F.; Destine, B., and Viard, F., 2002. Population structure of an endangered species living in contrasted habitats: *Parnassia palustris* (Saxifragaceae). *Molecular Ecology,* 11, 979–990.

Bonte, D.; Maelfait, J.-P., and Lens, L., 2006. Sand dynamics in coastal dune landscapes constrain diversity and life-history characteristics of spiders. *Journal of Applied Ecology,* 43, 735–747.

Borgen, L. and Hultgård, U.M., 2003. *Parnassia palustris:* a genetically diverse species in Scandinavia. *Botanical Journal of the Linnean Society,* 142, 347–372.

Bossuyt, B., 2007. Genetic rescue in an isolated metapopulation of a naturally fragmented plant species, *Parnassia palustris*. *Conservation Biology,* 21, 832–841.

Bossuyt, B. and Honnay, O., 2006. Interactions between plant life span, seed dispersal capacity and fecundity determine metapopulation viability in a dynamic landscape. *Landscape Ecology,* 21, 1195–1205.

Bossuyt, B.; Honnay, O., and Hermy, M., 2003. An island biogeographical view of the successional pathway in wet dune slacks. *Journal of Vegetation Science,* 14, 781–788.

Bouman, F.; Boesewinkel, D.; Bregman, R.; Devente, N., and Oostermeijer, G., 2000. Verspreiding van zaden. Utrecht, The Netherlands: Stichting Uitgeverij van de Koninklijke Nederlandse Natuurhistorische Vereniging. 240p.

Ellstrand, N.C. and Elam, D.R., 1993. Population genetic consequences of small population-size—implications for plant conservation. *Annual Review of Ecology and Systematics,* 24, 217–242.

Ennos, R.A. 1994. Estimating the relative rates of pollen and seed migration among plant populations. *Heredity,* 72, 250–259.

Hamrick, J.L. and Godt, M.J.W., 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London, Biological Sciences,* 351, 1291–1298.

Heidinger, I.; Hein, S., and Bonte, D., 2010. Patch connectivity and sand dynamics affect dispersal-related morphology of the blue-winged grasshopper *Oedipoda caerulescens* in coastal grey dunes. *Insect Conservation and Diversity,* 3, 205–212.

Holderegger, R. and Wagner, H.H., 2008. Landscape genetics. *Bioscience,* 58, 199–207.

Honnay, O. and Jacquemyn, H., 2007. Susceptibility of common and rare plant species to genetic consequences of habitat fragmentation. *Conservation Biology,* 21, 823–831.

Honnay, O.; Jacquemyn, H.; Van Looy, K.; Vandepitte, K., and Breyne, P., 2009. Temporal and spatial genetic variation in a metapopulation of the annual *Erysimum cheiranthoides* on stony river banks. *Journal of Ecology,* 97, 131–141.

Kondo, T.; Nakagoshi, N., and Isagi, Y., 2009. Shaping of genetic structure along Pleistocene and modern river systems in the hydrochorous riparian Azalea, *Rhododendron ripense* (Ericaceae). *American Journal of Botany,* 96, 1532–1543.

Kudoh, H. and Whigham, D.F., 1997. Microgeographic genetic structure and gene flow in *Hibiscus moscheutos* (Malvaceae) populations. *American Journal of Botany,* 84, 1285–1293.

Lambeets, K.; Breyne, P., and Bonte, D., 2010. Spatial genetic variation of a riparian wolf spider *Pardosa agricola* (Thorell, 1856) on lowland river banks: the importance of functional connectivity in linear spatial systems. *Biological Conservation,* 143, 660–668.

Leimu, R.; Mutikainen, P.; Koricheva, J., and Fischer M., 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology,* 94, 942–952.

Lynch, M. and Milligan, B.G., 1994. Analysis of population genetic structure with RAPD markers. *Molecular Ecology,* 3, 91–99.

Maes, D. and Bonte, D., 2006. Using distribution patterns of five threatened invertebrates in a highly fragmented dune landscape to develop a multispecies conservation approach. *Biological Conservation,* 133, 490–499.

Manel, S.; Schwartz, M.K.; Luikart, G., and Taberlet, P. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution,* 18, 189–197.

Martens, P. 1936. Pollination et biologie florare chez *Parnassia palustris* L. *Bulletin of the Societ'e Royale Botanique de Belgique,* 68, 183–231.

Nybom, H. and Bartish, I.V., 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution, and Systematics,* 3: 93–114.

Peakall, R. and Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes,* 6, 288–295.

Provoost, S.; Hoffmann, M.; Bonte, D., and Leten, M., 2004. Landschap en beheer van kustduinen. *In:* Hermy, M.; De Blust, G., and Slootmakers, M. (eds.), *Natuurbeheer*. Leuven: Davidsfonds i.s.m. Argus, Natuurpunt en Instituut voor Natuurbehoud.

Provoost, S.; Jones, M.L., and Edmondson, S.E. 2009. Changes in landscape and vegetation of coastal dunes in northwest Europe: a review. *Journal of Coastal Conservation*. DOI: 10.1007/s11852-009-0068-5

Sandvik, S.M. and Totland, O., 2003. Quantitative importance of staminodes for female reproductive success in *Parnassia palustris* under contrasting environmental conditions. *Canadian Journal of Botany—Revue Canadienne De Botanique,* 81, 49–56.

Sork, V.L.; Nason, J.; Campbell, D.R., and Fernandez, J.F., 1999. Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology and Evolution,* 14, 219–224.

Storfer, A.; Murphy, M.A.; Evans, J.S.; Goldberg, C.S.; Robinson, S.; Spear, S.F.; Dezzani, R.; Delmelle, E.; Vierling, L., and Waits, L.P., 2007. Putting the "landscape" in landscape genetics. *Heredity,* 98, 128–142.

Vandegehuchte, M.L.; de la Peña, E., and Bonte, D., 2010. Relative importance of biotic and abiotic soil components to plant growth and insect herbivore population dynamics. *PloS One,* 5, e11174.

Van Looy, K.; Jacquemyn, H.; Breyne, P., and Honnay, O., 2009. Effects of flood events on the genetic structure of riparian populations of the grassland plant *Origanum vulgare*. *Biological Conservation,* 142, 870–878.

Vekemans, X.; Beauwens, T.; Lemaire, M., and Roldan-Ruiz, I., 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology,* 11, 139–151.

Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; van de Lee, T.; Hornes, M.; Frijters, A.; Pot, J.; Peleman, J.; Kuiper, M., and Zabeau M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research,* 23(21), 4407–4414.

Wratten, S.D.; Bowie, M.H.; Hickman, J.M.; Evans, A.M.; Sedcole, J.R., and Tylianakis, J.M., 2003. Field boundaries as barriers to movement of hover flies (Diptera : Syrphidae) in cultivated land. *Oecologia,* 134, 605–611.

Young, A.; Boyle, T., and Brown, T. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution,* 11, 413–418.